FIG. 1

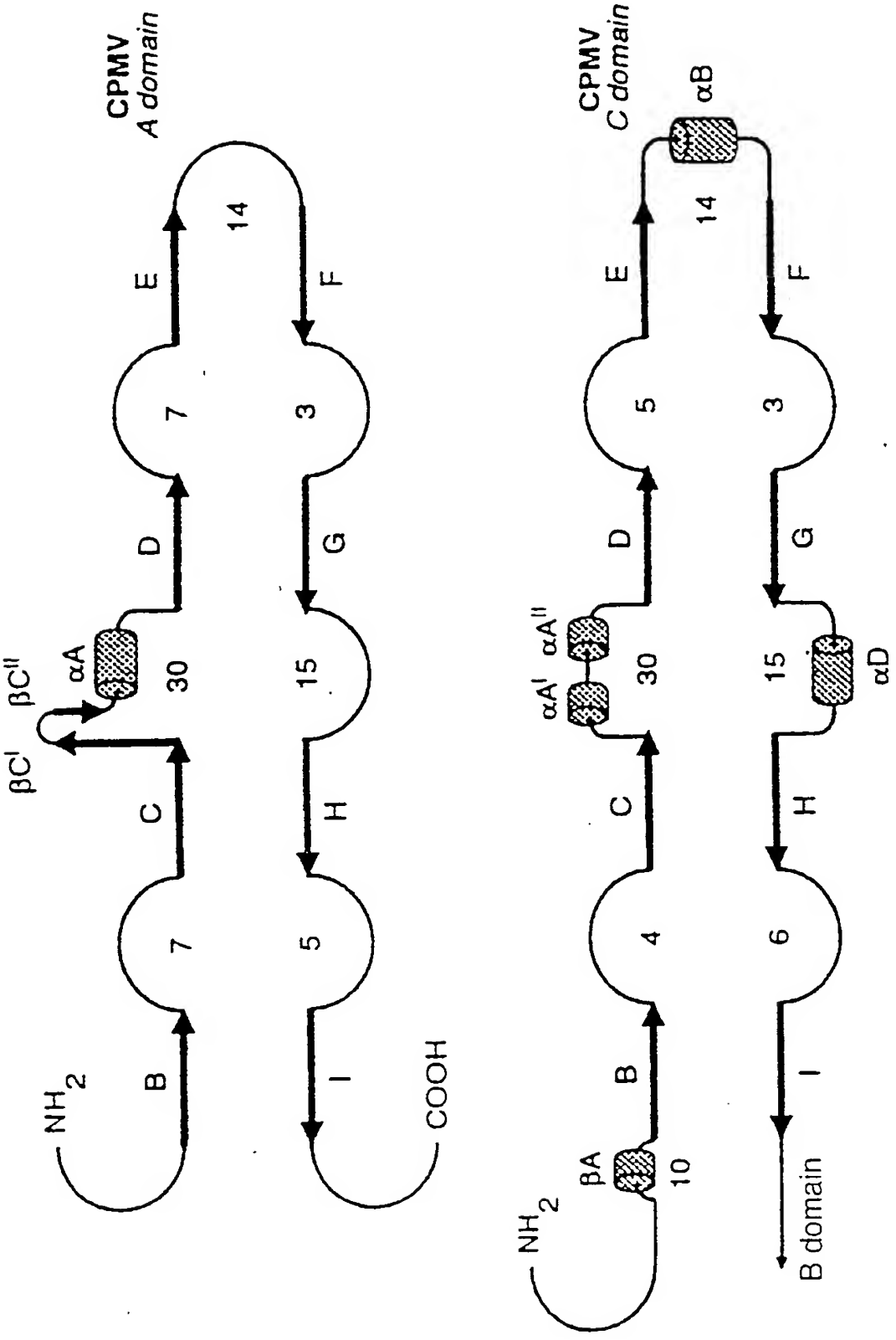

CPMV
A domain
NH2
B
7
C
βC'
βC''
αA
30
D
7
E
14
F
3
G
15
H
5
I
COOH
CPMV
C domain
NH2
βA
10
B
4
C
αA'
αA''
30
D
5
E
14
αB
F
3
G
15
αD
H
6
I
B domain

FIG. 1(contd.)

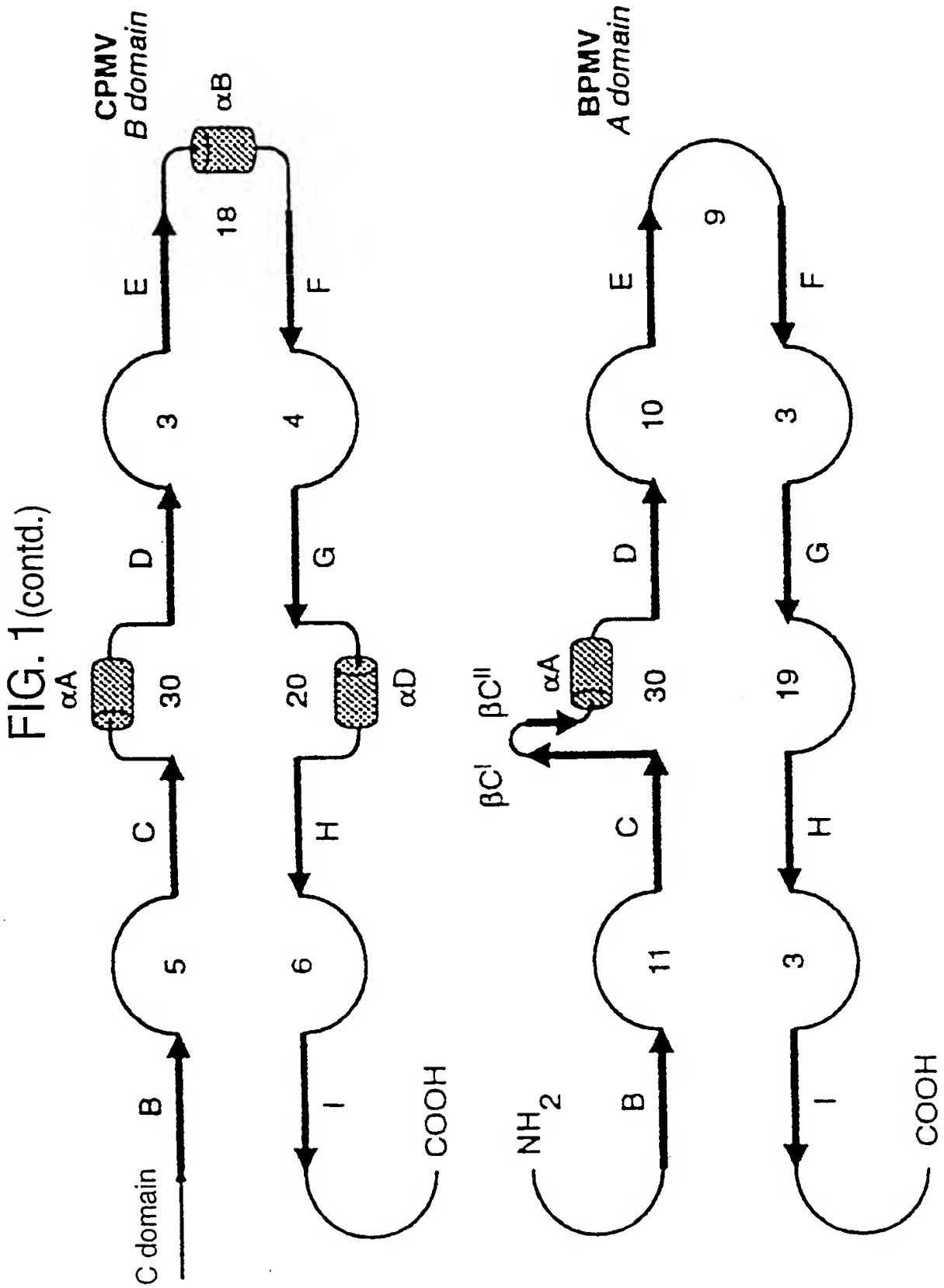

CPMV
B domain
αB
18
E
F
3
4
D
G
αA
30
20
αD
C
H
5
6
B
I
C domain
COOH
BPMV
A domain
9
E
F
10
3
D
G
αA
βC^II
βC^I
30
19
C
H
11
3
NH2
B
I
COOH

FIG. 1(contd.)

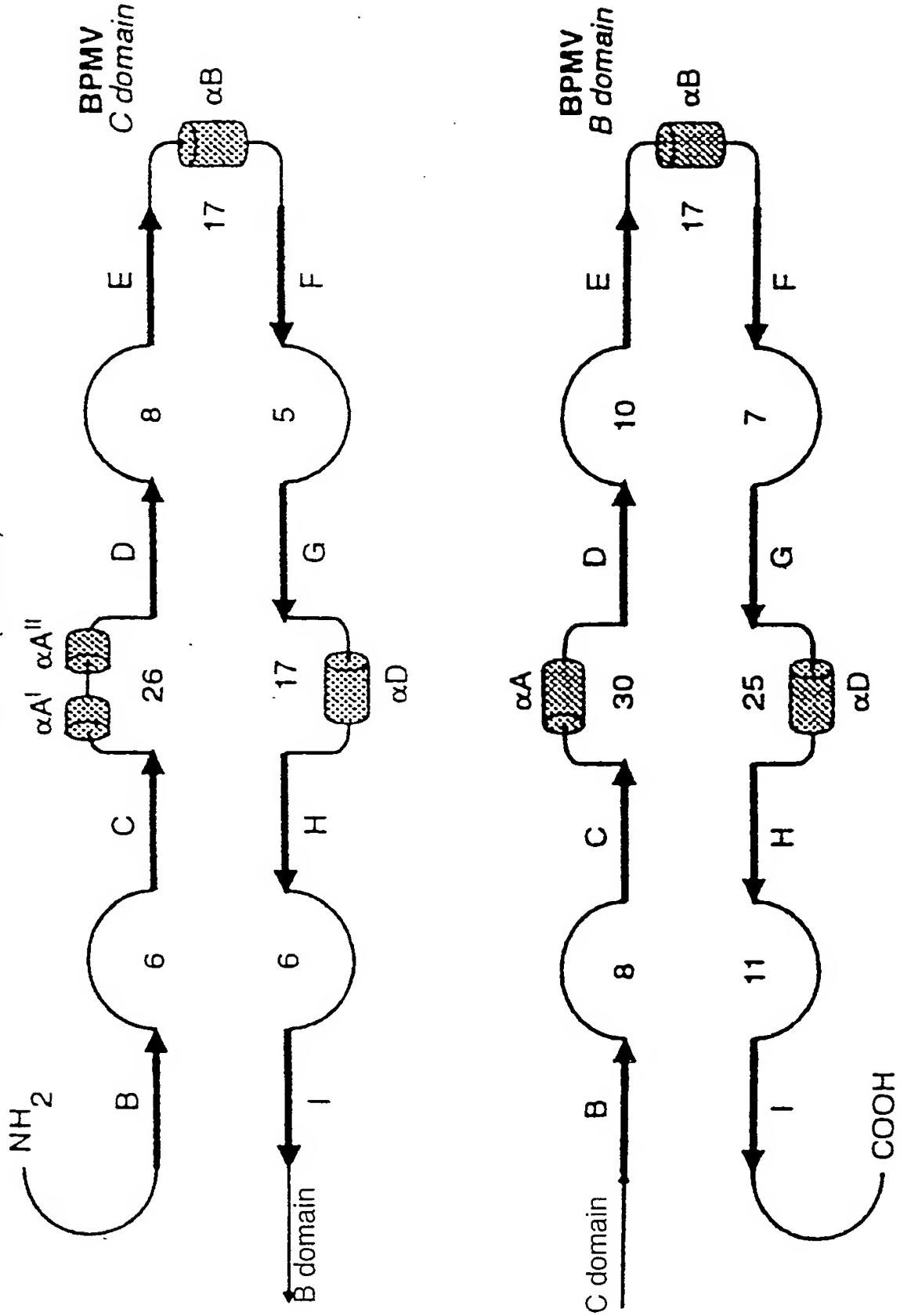

BPMV
C domain
αB
17
E
F
8
5
D
G
αA' αA''
26
17
αD
C
H
6
6
NH2
B
I
B domain
BPMV
B domain
αB
17
E
F
10
7
D
G
αA
30
25
αD
C
H
8
11
B
I
C domain
COOH

FIG. 1(contd.)

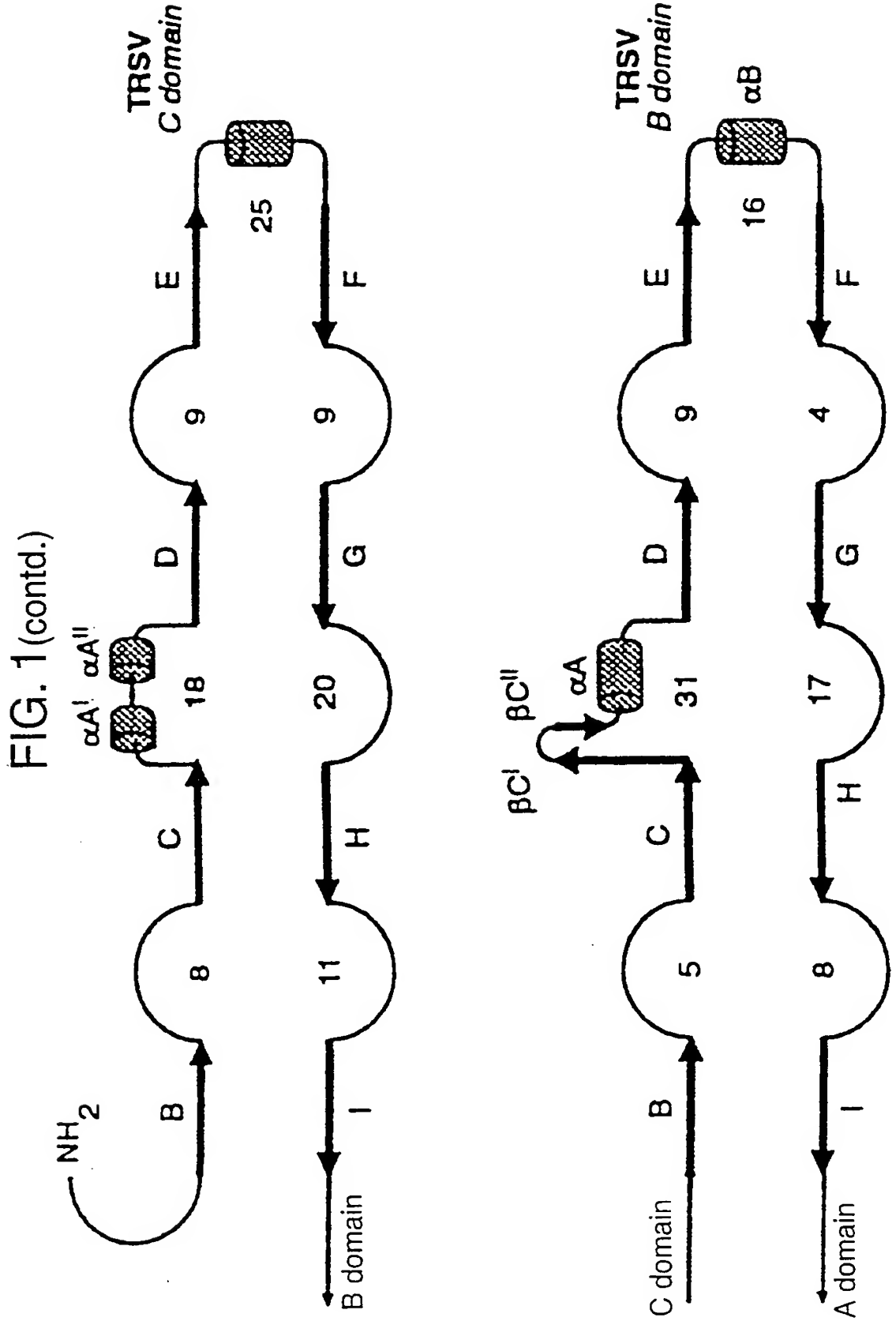

TRSV
C domain
TRSV
B domain
αB
αA' αA''
αA
βC' βC''
NH2
B
C
D
E
F
G
H
I
8
18
9
25
9
20
11
16
9
4
17
8
31
5
B domain
C domain
A domain

FIG. 1(contd.)

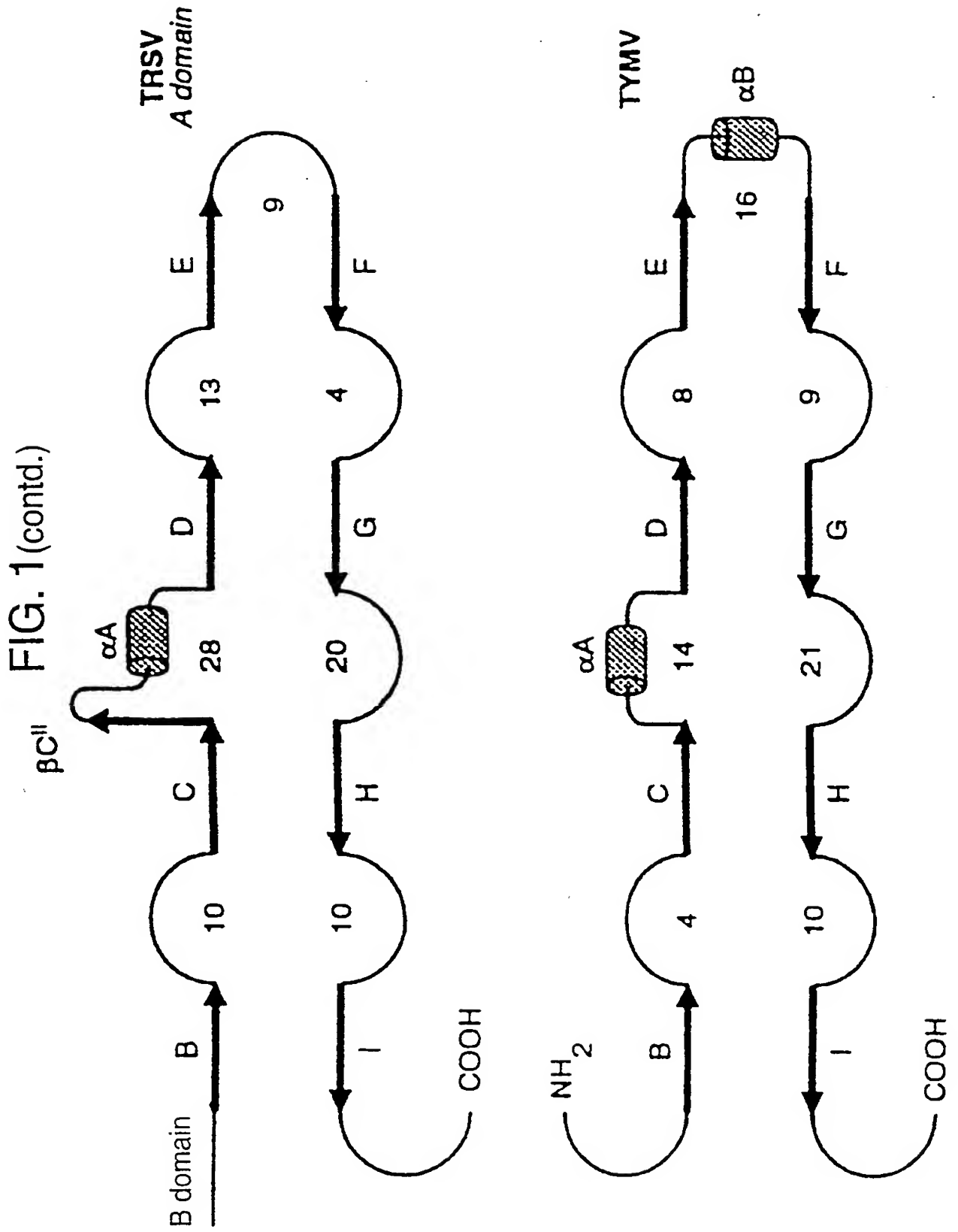
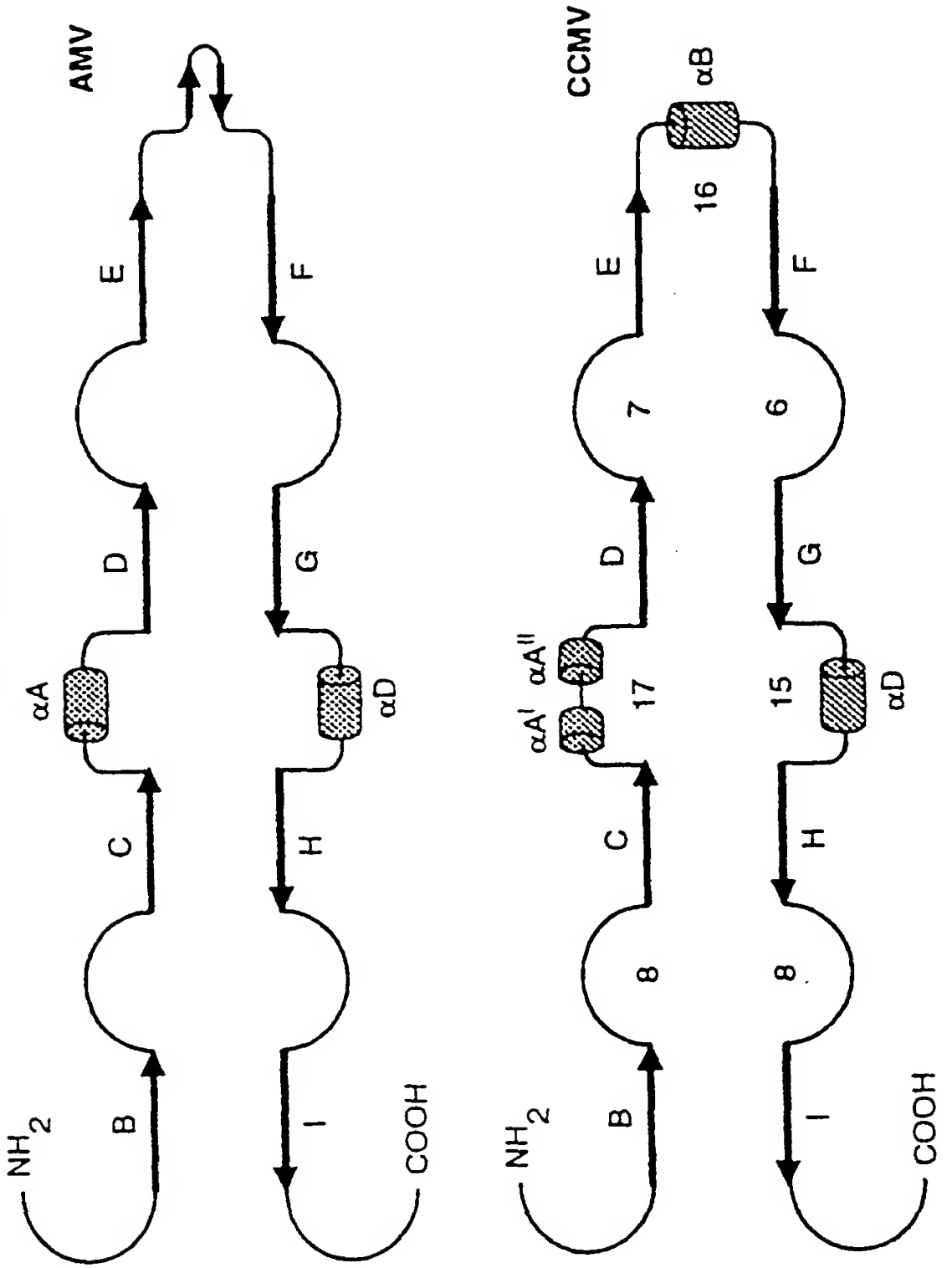

TRSV
A domain
B domain
B
10
C
βC''
αA
28
D
13
E
9
F
4
G
20
H
10
I
COOH
TYMV
NH2
B
4
C
αA
14
D
8
E
αB
16
F
9
G
21
H
10
I
COOH

FIG. 1(contd.)



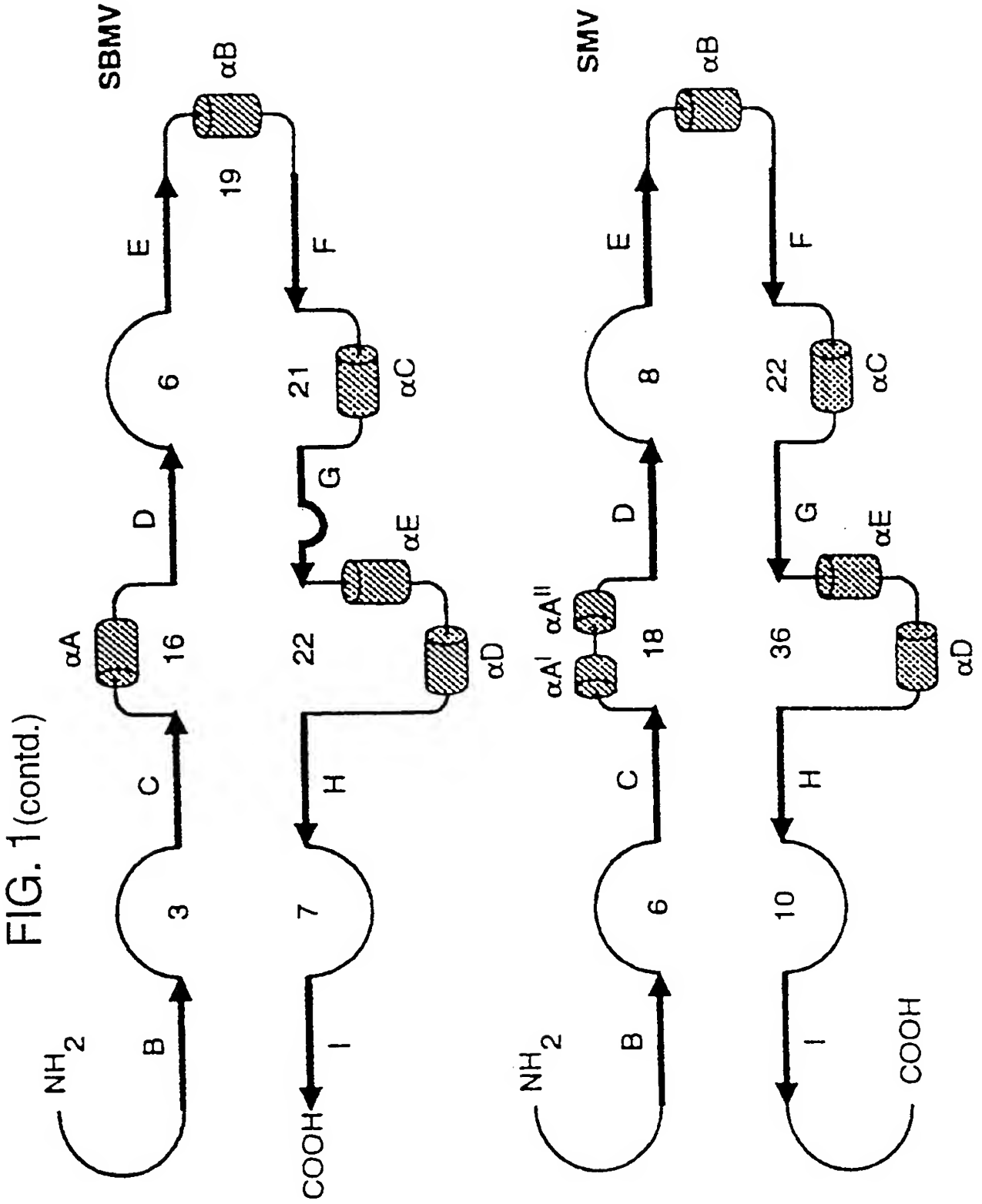

SBMV
αB
19
E
F
6
21
αC
G
D
αE
αA
16
22
αD
C
H
3
7
NH2
B
I
COOH
SMV
αB
E
F
8
22
αC
G
D
αE
αA' αA''
18
36
αD
C
H
6
10
NH2
B
I
COOH

FIG. 1(contd.)

FIG. 1(contd.)

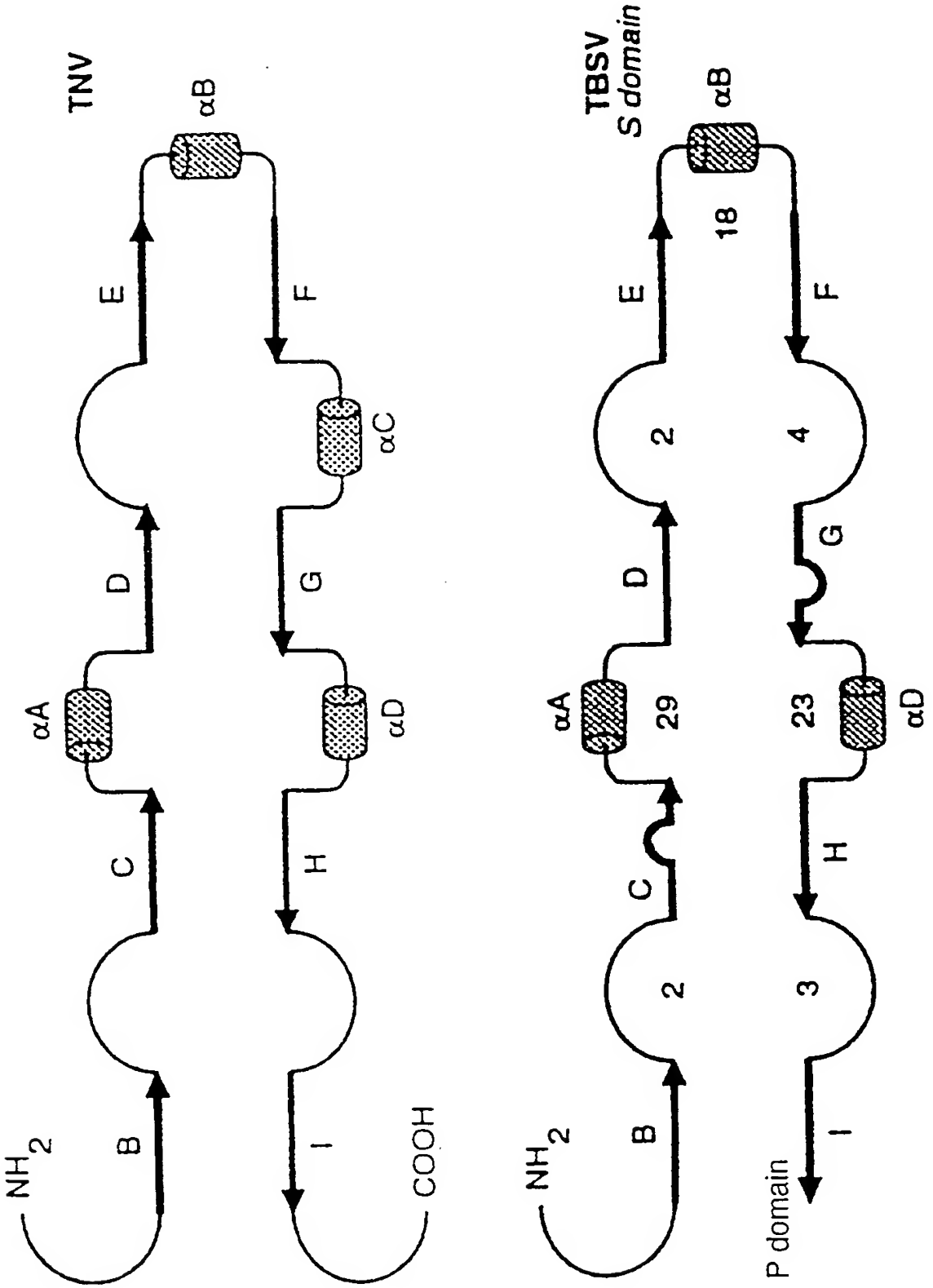

TNV
αB
E
F
αC
D
G
αA
αD
C
H
NH2
B
I
COOH
TBSV
S domain
αB
18
E
F
2
4
D
G
αA
29
23
αD
C
H
2
3
NH2
B
I
P domain

FIG. 1(contd.)

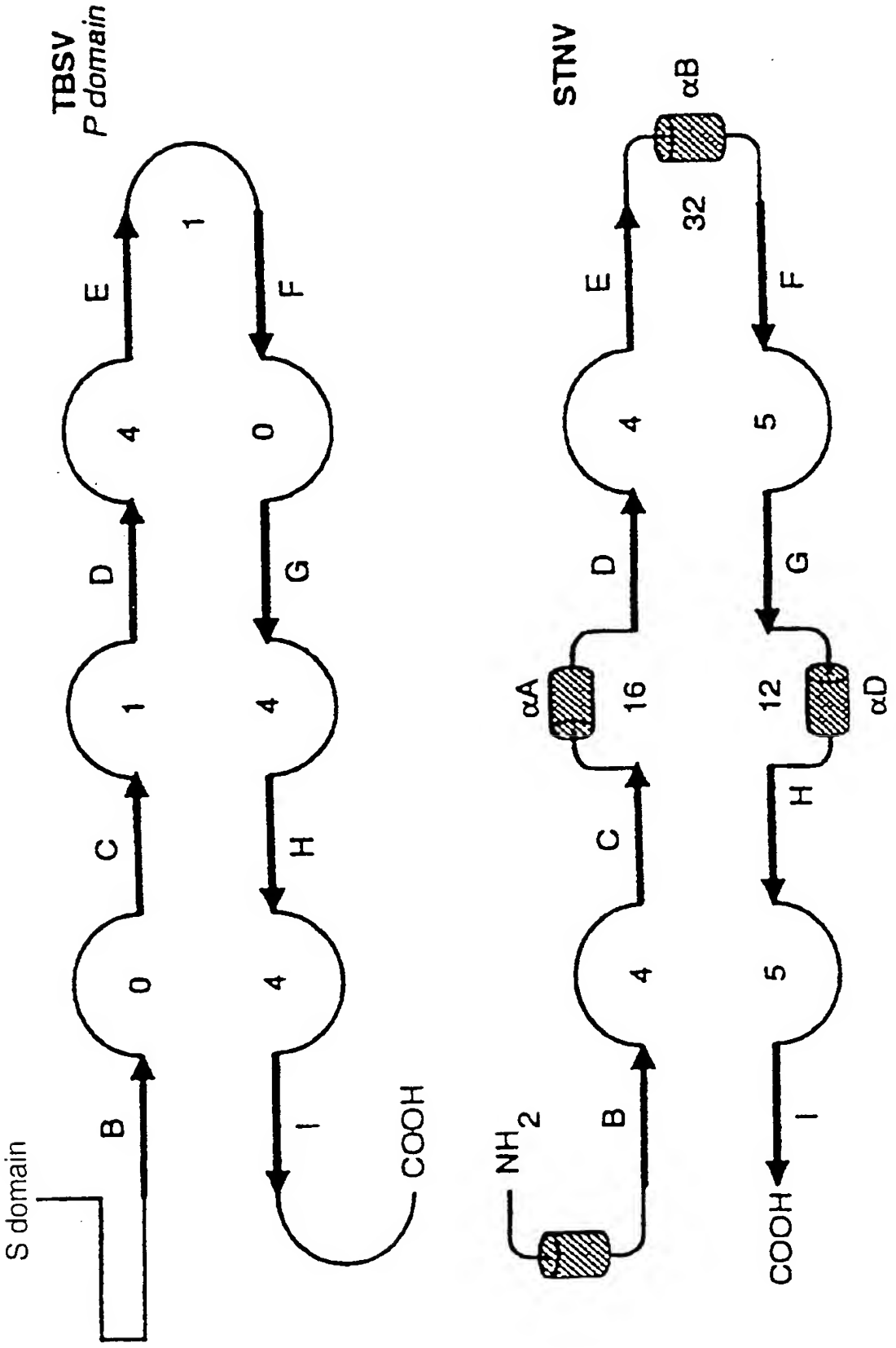

TBSV
P domain
S domain
B
0
C
1
D
4
E
1
F
0
G
4
H
4
I
COOH
STNV
αB
32
E
4
F
5
G
αA
16
D
12
αD
C
H
NH2
B
4
5
I
COOH

FIG. 1(contd.)

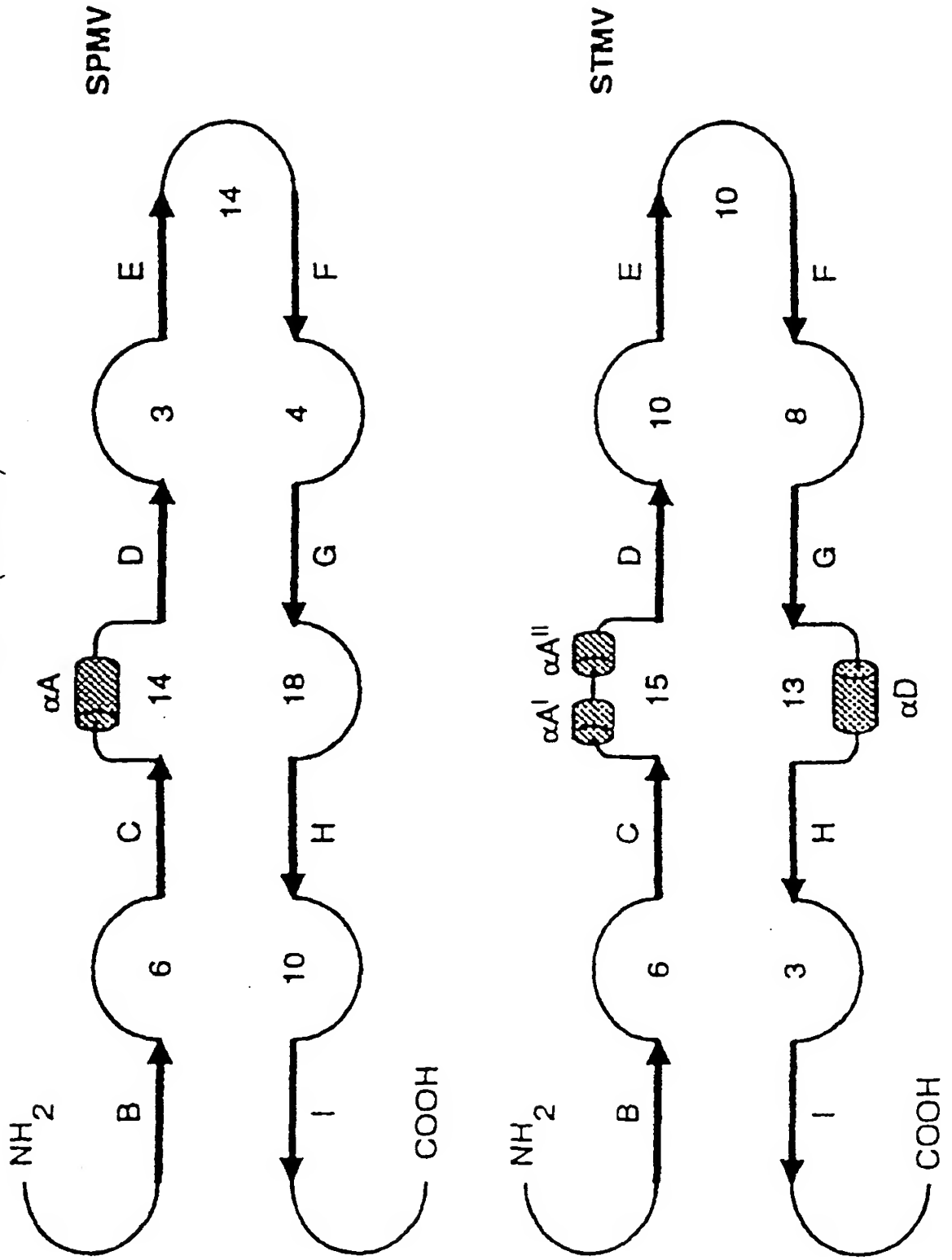


FIG. 2A

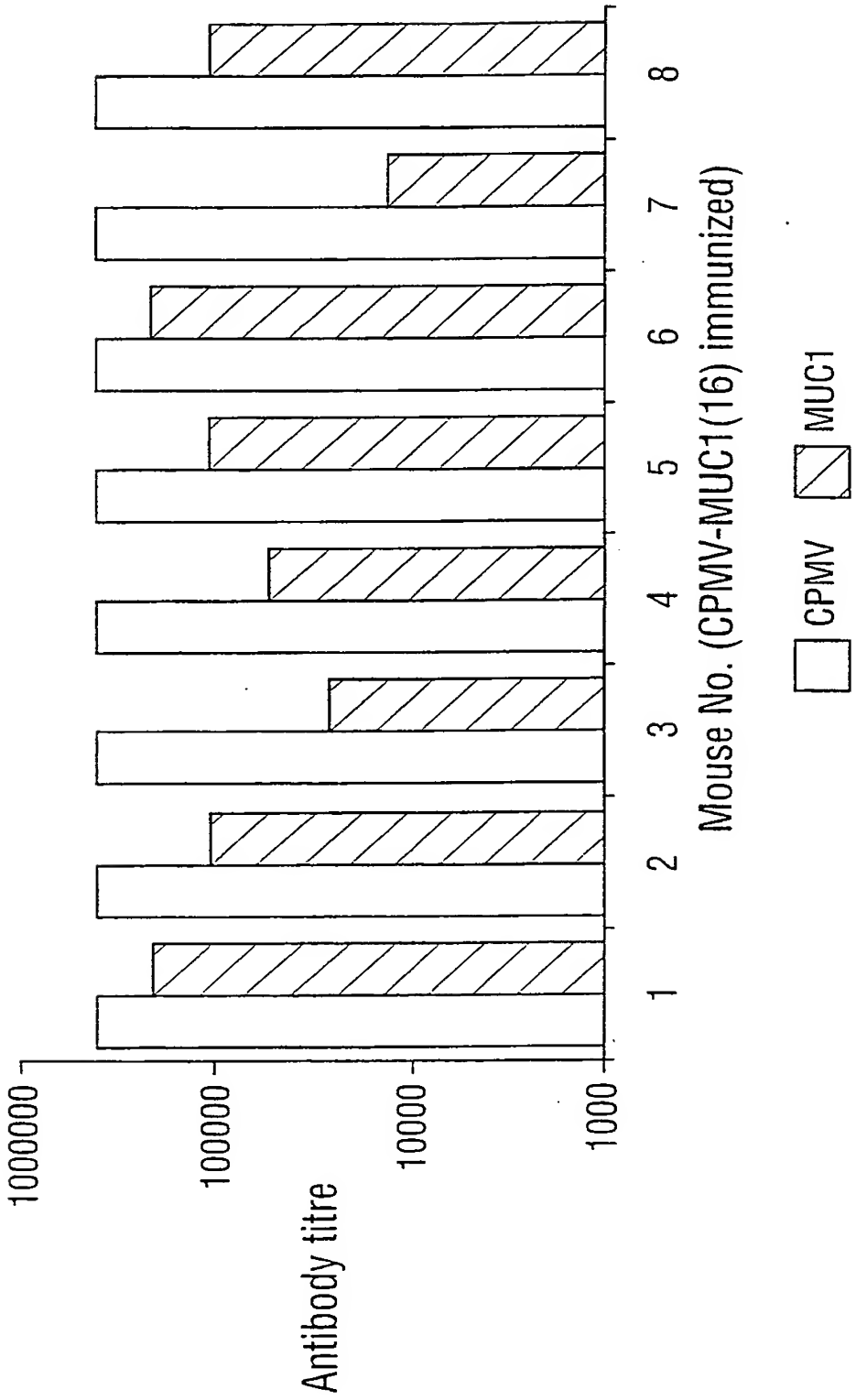

1000000
100000
10000
1000
Antibody titre
1
2
3
4
5
6
7
8
Mouse No. (CPMV-MUC1(16) immunized)
CPMV
MUC1

FIG. 2B

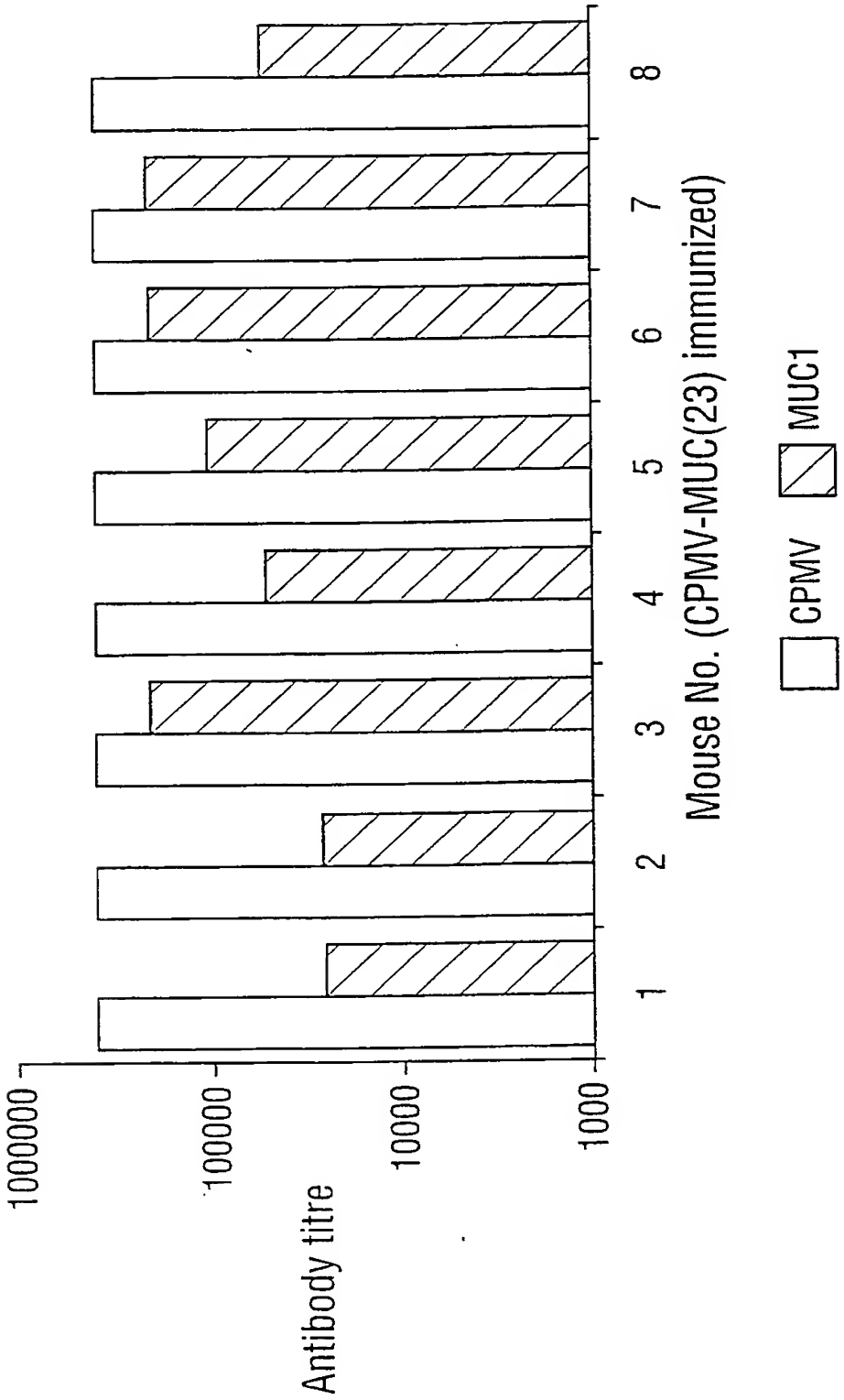

Antibody titre
1000000
100000
10000
1000
1
2
3
4
5
6
7
8
Mouse No. (CPMV-MUC(23) immunized)
CPMV
MUC1

FIG. 2C

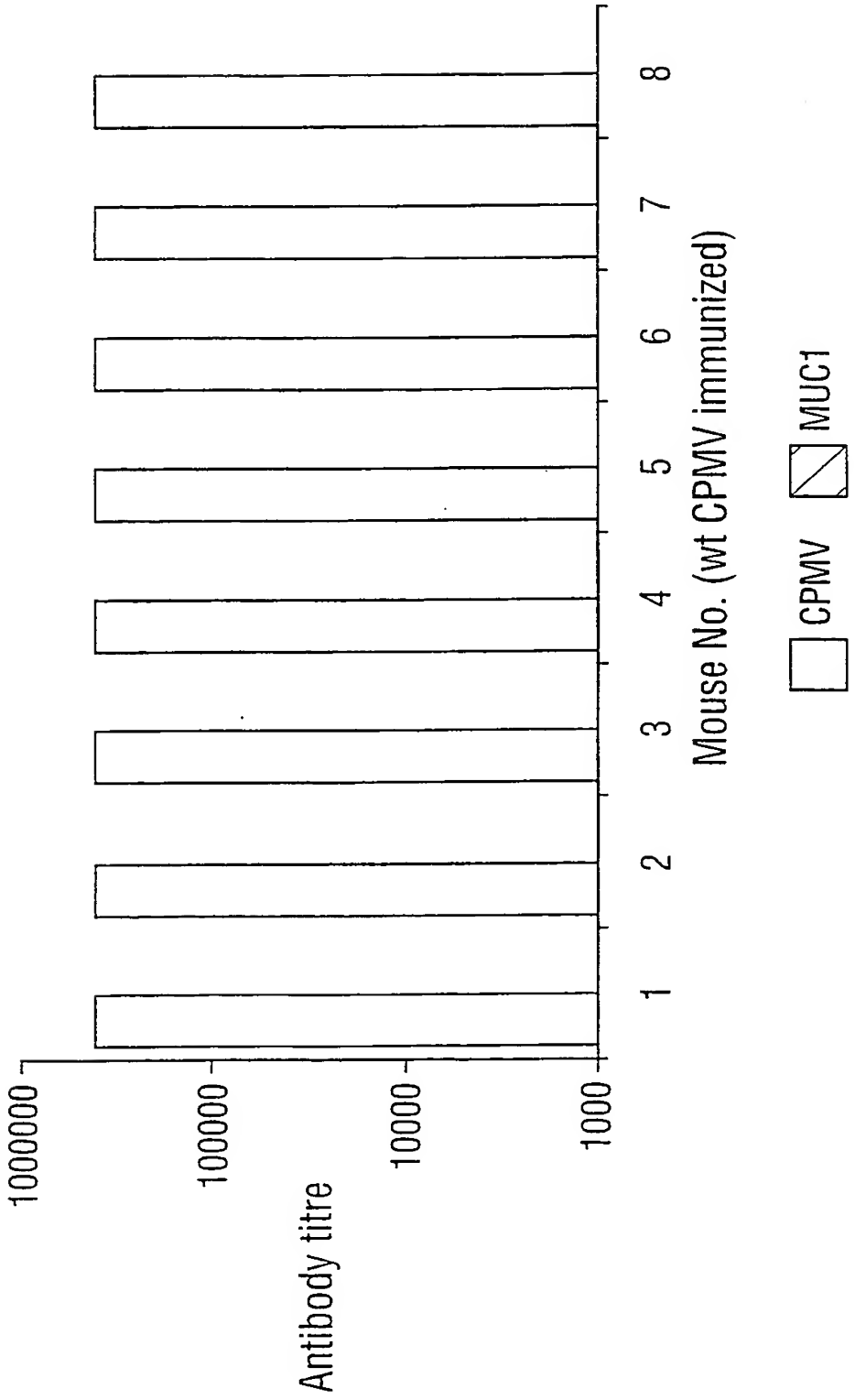

1000000
100000
10000
1000
Antibody titre
1
2
3
4
5
6
7
8
Mouse No. (wt CPMV immunized)
CPMV
MUC1

FIG. 3A

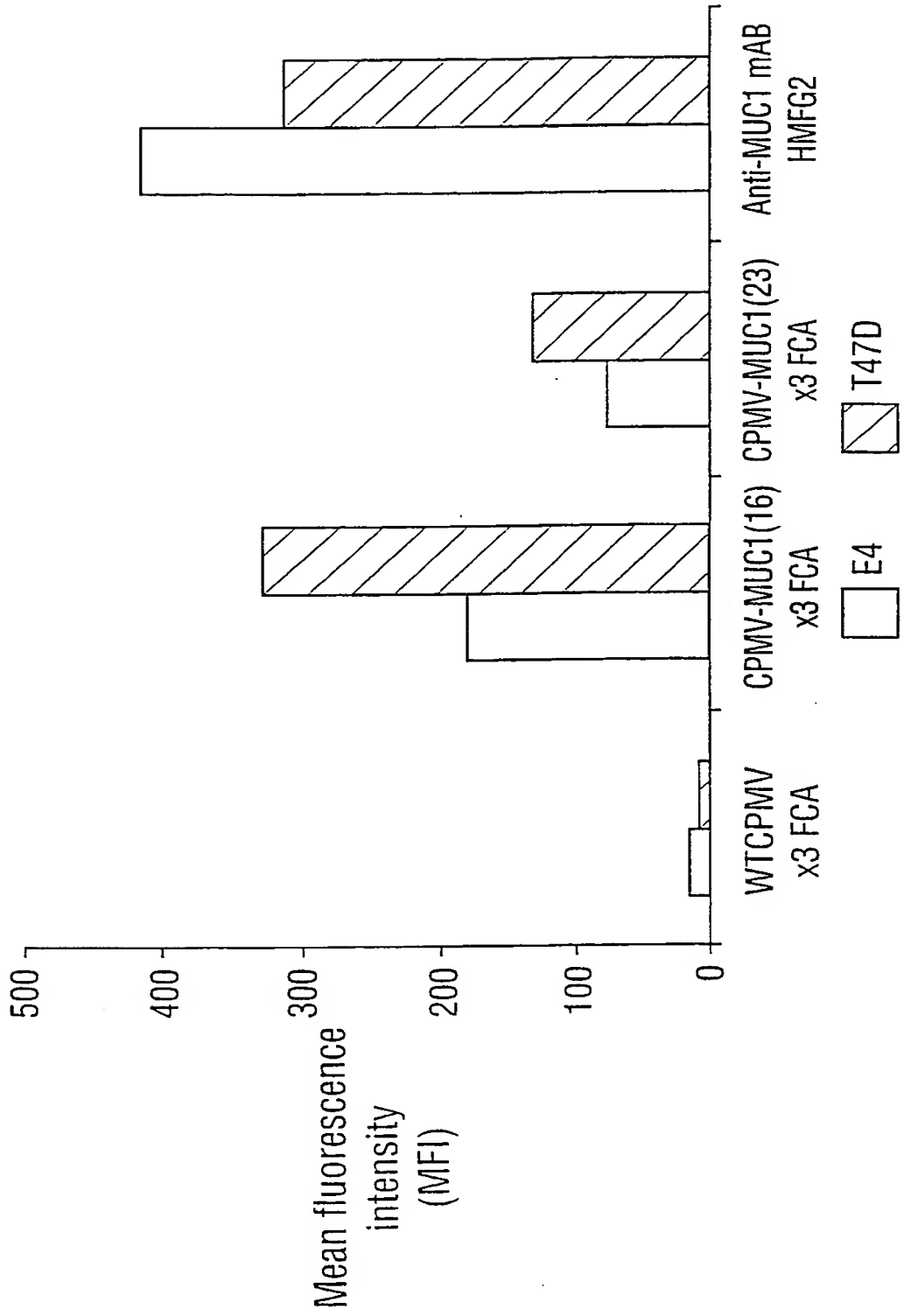

Mean fluorescence intensity (MFI)
500
400
300
200
100
0
WTCPMV x3 FCA
CPMV-MUC1(16) x3 FCA
CPMV-MUC1(23) x3 FCA
Anti-MUC1 mAB HMFG2
E4
T47D

FIG. 3B

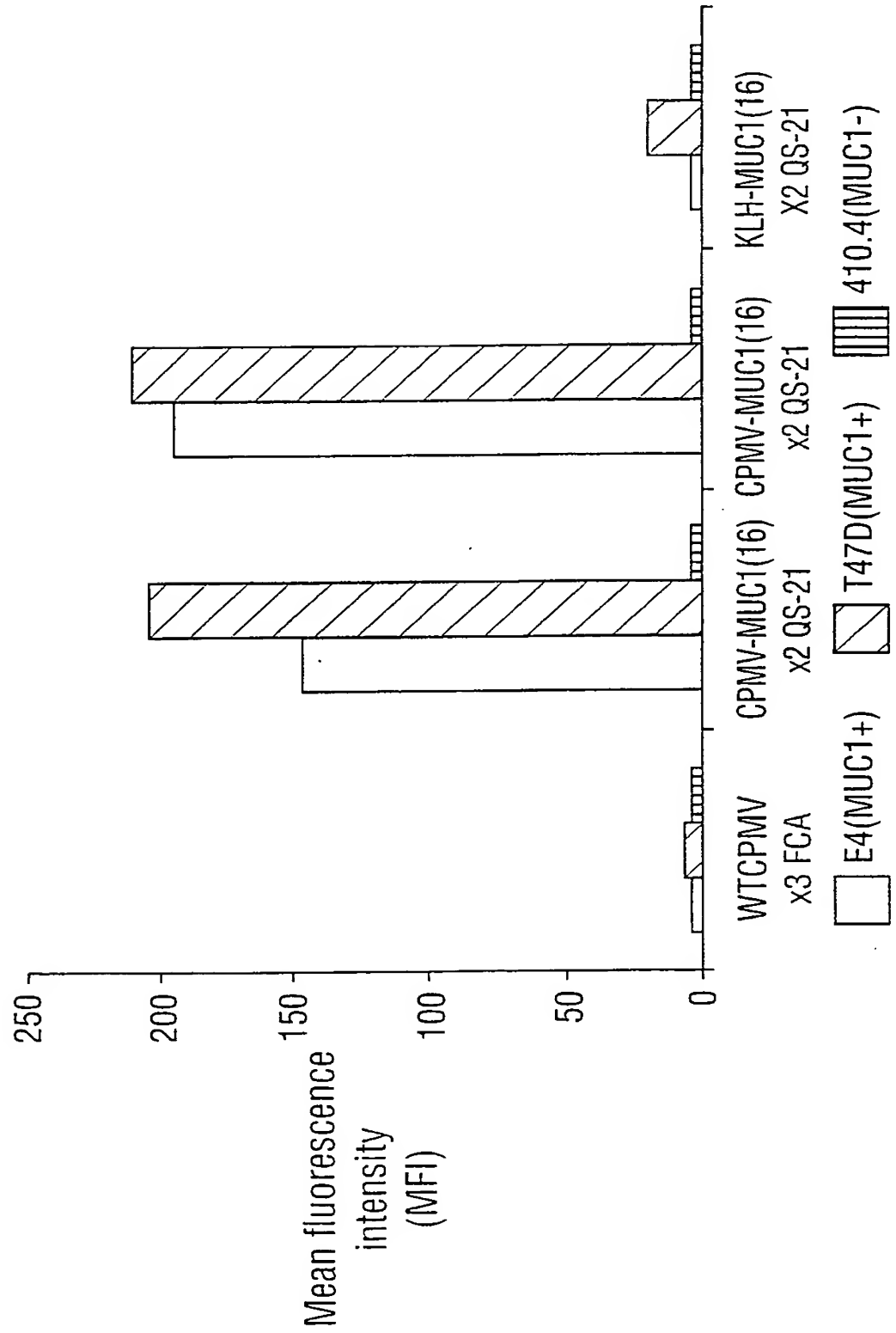

Mean fluorescence intensity (MFI)
250
200
150
100
50
0
WTCPMV x3 FCA
CPMV-MUC1(16) x2 QS-21
CPMV-MUC1(16) x2 QS-21
KLH-MUC1(16) X2 QS-21
E4(MUC1+)
T47D(MUC1+)
410.4(MUC1-)

FIG. 4

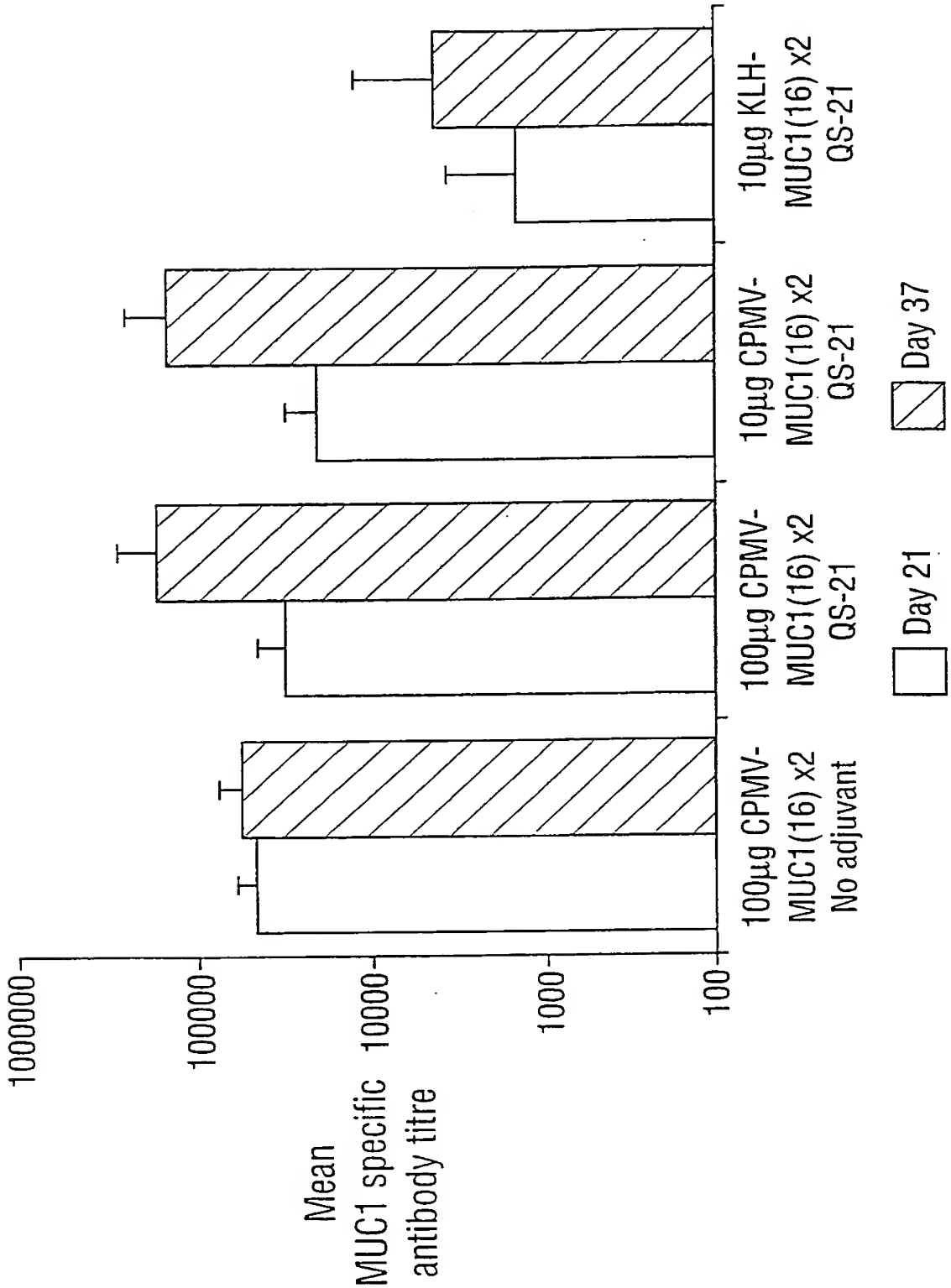

Mean MUC1 specific antibody titre
1000000
100000
10000
1000
100
100µg CPMV-MUC1(16) x2 No adjuvant
100µg CPMV-MUC1(16) x2 QS-21
10µg CPMV-MUC1(16) x2 QS-21
10µg KLH-MUC1(16) x2 QS-21
Day 21
Day 37

FIG. 5

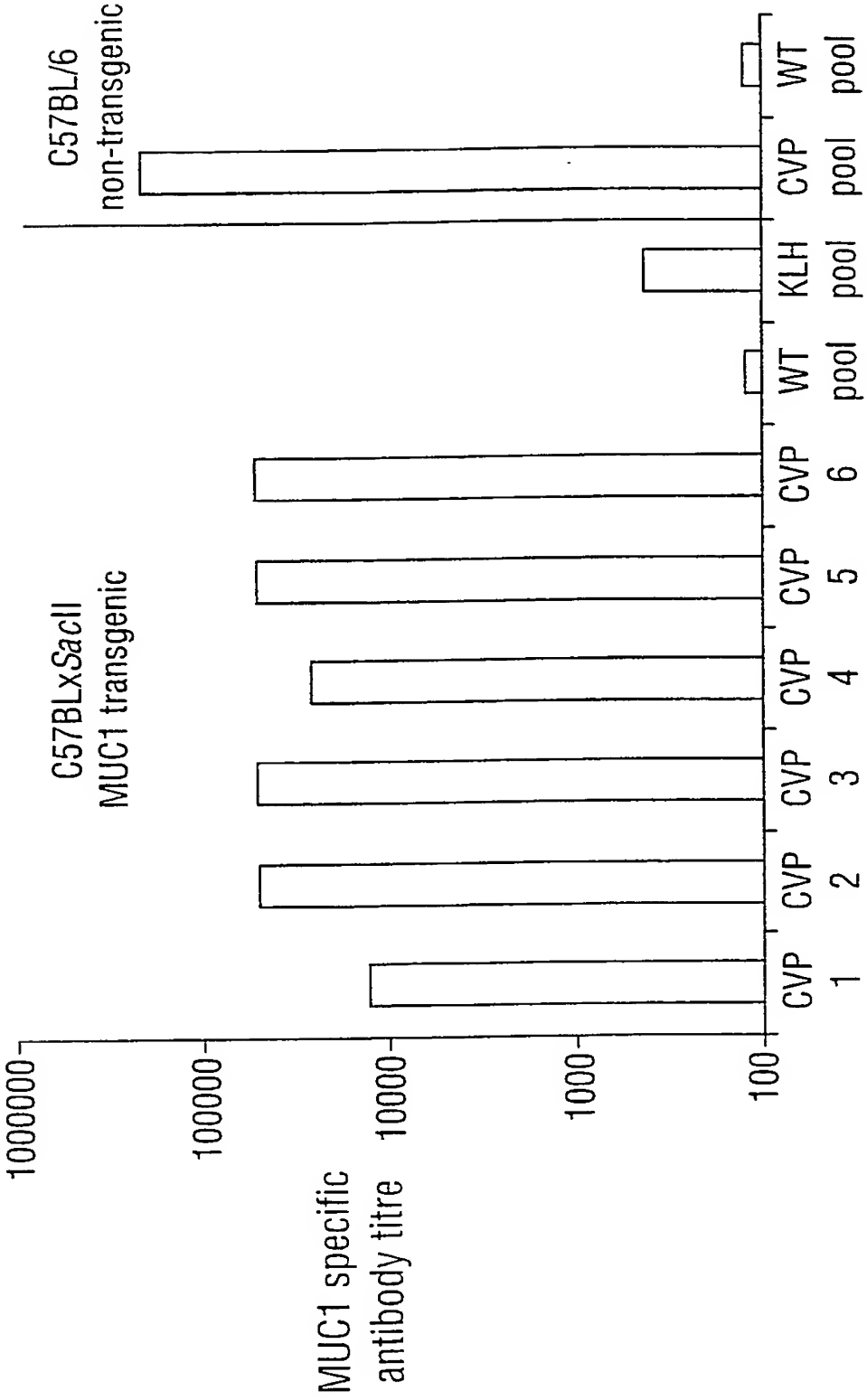

C57BL/6
non-transgenic
C57BLxSacII
MUC1 transgenic
MUC1 specific
antibody titre
1000000
100000
10000
1000
100
CVP 1
CVP 2
CVP 3
CVP 4
CVP 5
CVP 6
WT pool
KLH pool
CVP pool
WT pool

FIG. 6

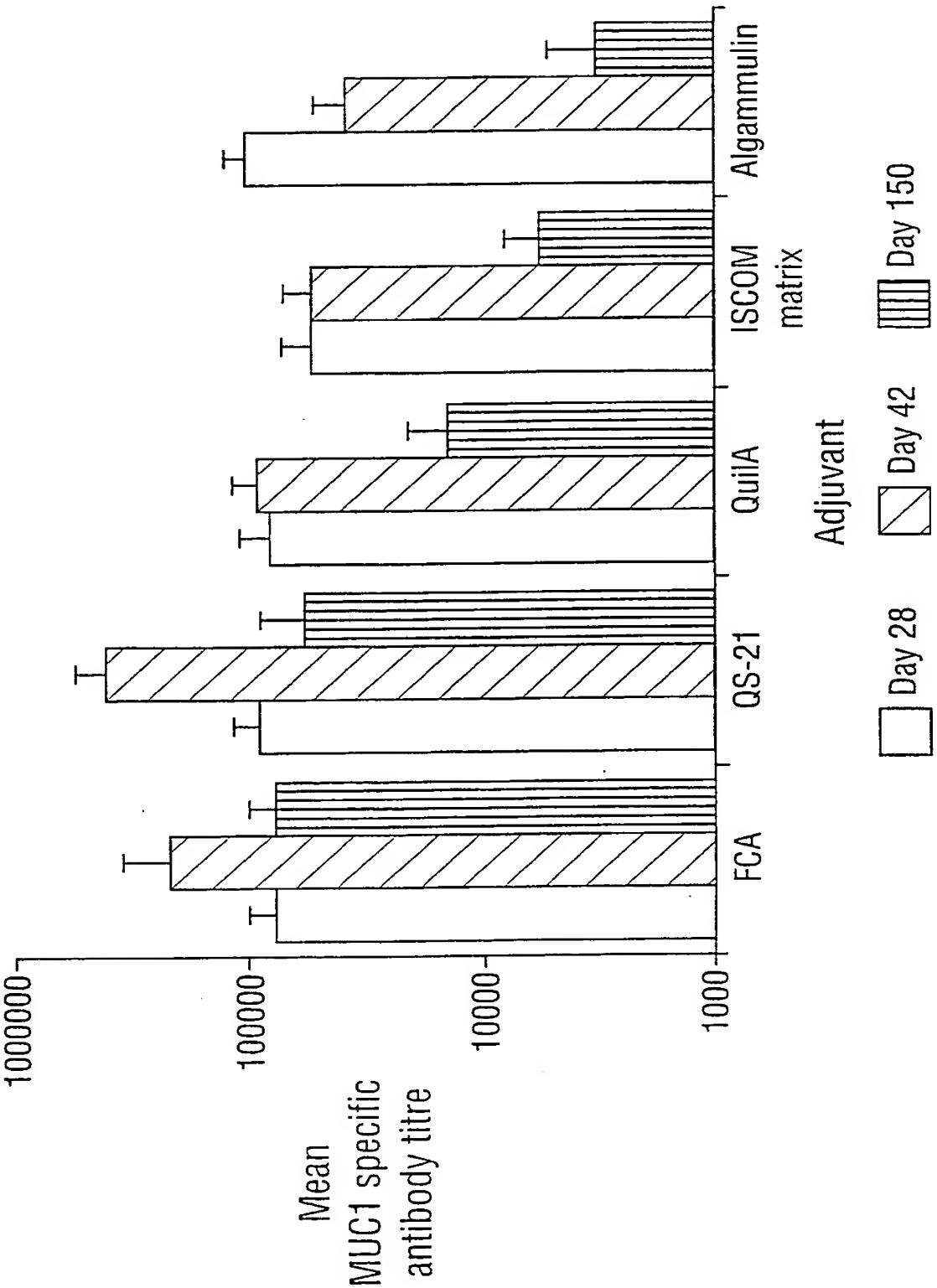

1000000
100000
10000
1000
Mean MUC1 specific antibody titre
FCA
QS-21
QuilA
ISCOM matrix
Algammulin
Adjuvant
Day 28
Day 42
Day 150

FIG. 7A

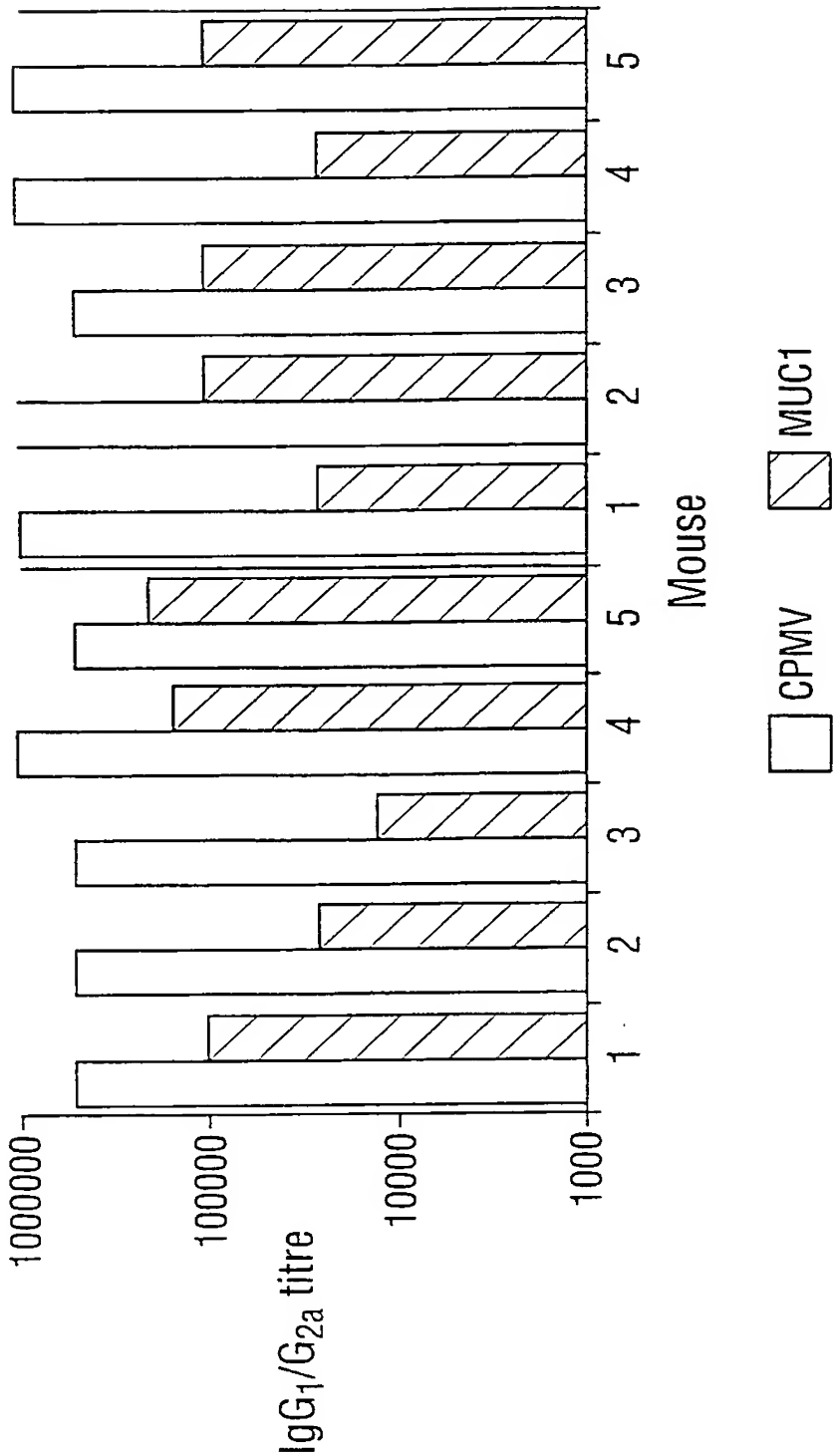

CPMV-MUC1(16) No adjuvant
CPMV-MUC1(16) + CT
IgG1/G2a titre
1000000
100000
10000
1000
1
2
3
4
5
Mouse
CPMV
MUC1

FIG. 7B

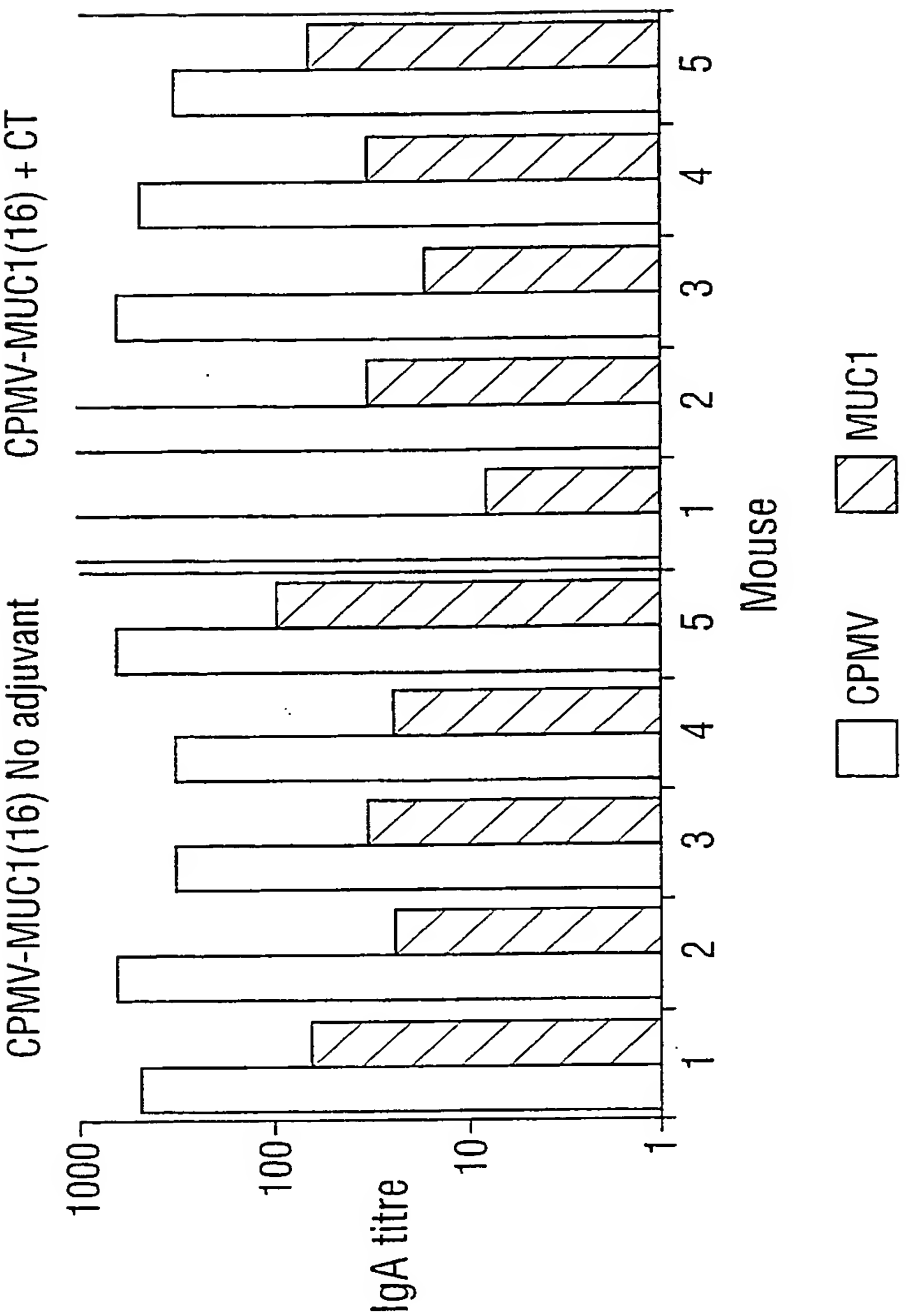

CPMV-MUC1(16) No adjuvant
CPMV-MUC1(16) + CT
IgA titre
1000
100
10
1
1
2
3
4
5
1
2
3
4
5
Mouse
CPMV
MUC1

FIG. 8(a)

Sequence of SBMV Coat Protein Spanning The Potential Insertion Site With Introduced Base Changes and New Restriction Sites: (sequence starts at nt 3955)

```
M  E  G  G  S  S  K  T  A  V  N  T  G
ATGGAAGGAGGATCATCTAAGACTGCTGTGAACACTGGG
            ↓                 ↓
          GGATCC            GTTAAC
          BamH I            Hpa I
```

FIG. 8(b)

Series of Sequences to be Inserted Between the Restriction Sites to Insert the MUC1(16) Epitope at Various Locations

```
G  V  T  S  A  P  D  T  R  P  A  P  G  S  T  A
GGTGTTACTTCTGCTCCTGATACTAGACCTGCTCCTGGTTCTACTGCT
CCACAATGAAGACGACCACTATGATCTGGACGAGGACCAAGATGACGA
```

GATCC	TCTAAGACTGCTGTT
G	AGATTCTGACGACAA
GATCCTCT	AAGACTGCTGTT
GAGA	TTCTGACGACAA
GATCCTCTAAG	ACTGCTGTT
GAGATTC	TGACGACAA
GATCCTCTAAGACT	GCTGTT
GAGATTCTGA	CGACAA
GATCCTCTAAGACTGCT	GTT
GAGATTCTGACGA	CAA

FIG. 9

```
LTSV : NI---YAPARLTIAA-ANSSINIASVGTLYATYEVEL
SBMV : NIGNILVPARLVIAMEGGSSKTAVNTGRLYASYTIRL
SMV  : NIATDLVPARLVIALLDGSSSTAVAAGRIYASYTIQM
                ###########=############
                  βH    loop      βI
```

FIG. 13

```
                      220       230       240
          AA         |ASIVQKYVIDLGGTLTSFEGPSYLMPP
          PHD sec    | HHHHHEEEE   EEEE    EEEEE
          Rel sec    |145432244525515625586487624
detail :
          prH sec    |466655321111100000000000000
          prE sec    |101123456632246752212688753
          prL sec    |422221112246642237787311246
subset :  SUB sec    |..H......E.LL.EE.LLLL.EEE..
```

Abbreviations :

AA : amino acid sequence
H : helix
E : extended (sheet)
blank : other (loop)
PHD : Profile network prediction HeiDelberg
Rel : Reliability index of prediction (0-9)
prH : probability for assigning helix
prE : probability for assigning strand
prL : probability for assigning loop
SUB : a subset of the prediction, for all residues with an average expected accuracy of >82%

FIG. 10(a)

Sequence of LTSV Coat Protein Spanning The Potential Insertion Site With Introduced Base Changes and New Restriction Sites: (sequence starts at nt 3954)

```
I  A  A  A  N  S  S  I  N  I  A  S  V  G  T  L  Y
ATAGCCGCAGCTAACAGCTCCATAAACATAGCTAGTGTGGGTACTCTTTAT
      ↓                                    ↓
    CTGCAG                               GGTACC
    Pst I                                Kpn I
```

FIG. 10(b)

Series of Sequences to be Inserted Between the Restriction Sites to Insert the MUC1(16) Epitope at Various Locations.

```
G  V  T  S  A  P  D  T  R  P  A  P  G  S  T  A
GGTGTTACTTCTGCTCCTGATACTAGACCTGCTCCTGGTTCTACTGCT
CCACAATGAAGACGACCACTATGATCTGGACGAGGACCAAGATGACGA
```

GCTAACAGC	TCCATAAACATAGCTAGTGTGGGTAC
ACGTCGATTGTCG	AGGTATTTGTATCGATCACACC
GCTAACAGCTCC	ATAAACATAGCTAGTGTGGGTAC
ACGTCGATTGTCGAGG	TATTTGTATCGATCACACC
GCTAACAGCTCCATA	AACATAGCTAGTGTGGGTAC
ACGTCGATTGTCGAGGTAT	TTGTATCGATCACACC
GCTAACAGCTCCATAAAC	ATAGCTAGTGTGGGTAC
ACGTCGATTGTCGAGGTATTTG	TATCGATCACACC
GCTAACAGCTCCATAAACATA	GCTAGTGTGGGTAC
ACGTCGATTGTCGAGGTATTTGTAT	CGATCACACC
GCTAACAGCTCCATAAACATAGCT	AGTGTGGGTAC
ACGTCGATTGTCGAGGTATTTGTATCGA	TCACACC

FIG. 11

Lipman-Pearson alignment of RCNMV and TBSV coat protein sequences.

Lipman-Pearson Protein Alignment
Ktuple: 2; Gap Penalty: 4; Gap Length Penalty: 12

Seq1(1>389) tbsvtbs.PRO	Seq2(1>340) rcnmvdia.PRO	Similarity Index	Gap Number	Gap Length	Consensus Length
(64>387)	(8>338)	26.9	4	7	331

```
      ₣70        ₣80        ₣90        ₣100       ₣110       ₣120
KKQQMINHVGGTGGAIMAPVAVTRQLVGSKPKFTGRTSGSVTVTHREYLSQVNNSTGFQV
K.:Q. :. . T .: : .VA:. . .    . . ... : .: H : :  V .S. .:.
KSKQRSQPRNRTPNTSVKTVAIPFAKTQIIKTVNPPPKPARGILHTQLVMSVVGSVQMRT
  ₺10        ₺20        ₺30        ₺40        ₺50        ₺60
      ₣130       ₣140       ₣150       ₣160       ₣170       ₣180
NGGIVGNLLQLNPLNGTLFSWLPAIASNFDQYTFNSVVLHYVPLCSTTEVGRVAIYFDKD
N.G  .: ::LNP N :LF: L:  A:N:D Y :: .:.L:YVPL :. : GRVA: .D D
NNGKSNQRFRLNPSNPALFPTLAYEAANYDMYRLKKLTLRYVPLVTVQNSGRVAMIWDPD
  ₺70        ₺80        ₺90        ₺100       ₺110       ₺120
      ₣190       ₣200       ₣210       ₣220       ₣230       ₣240
SEDPEPADRVELANYSVLKETAPWAEAMLRVPTDKIKRFCDDSSTSDHKLIDLGQLGIAT
S:D:.P..R E::.YS   .TA ...  L :P:D:  RF .D::T D:KL:D:GQL :.T
SQDSAPQSRQEISAYSRSVSTAVYEKCSLTIPADNQWRFVADNTTVDRKLVDFGQLLFVT
  ₺130       ₺140       ₺150       ₺160       ₺170       ₺180
      ₣250       ₣260       ₣270       ₣280       ₣290          ₣300
YGGAGTNAVGDIFISYSVTLYFPQPTNTLLSTRRLDLAGALVTASGPGYLLVSR---TAT
.:G::. ..GDIF:...V.:  PQPT.:::  .  :DL:G:L.: .GP:YL: :    T::
HSGSDGIETGDIFLDCEVEFKGPQPTASIVQKTVIDLGGTLTSFEGPSYLMPPDAFITSS
  ₺190       ₺200       ₺210       ₺220       ₺230       ₺240
        ₣310       ₣320       ₣330       ₣340       ₣350
VLTMTFRATGTFVISGTYRCLTATTLGLAG--GVNVNSITVVDNIG-TDSAFFINCTVSN
 :.:   .:GT:::: .  C T:.:::::G  .:  :: :. :: . ..S  F.:..V :
SFGLFVDVAGTYLLTLVVTCSTTGSVTVGGNSTLVGDGRAAYGSSNYIASIVFTSSGVLS
  ₺250       ₺260       ₺270       ₺280       ₺290       ₺300
  ₣360       ₣370       ₣380
LPSVVTFT-STGITSATVHCVRATRQNDVSL
 .: V F: S:G::.. ::  R .: N.  L
TTPSVQFSGSSGVSRVQMNICRCKQGNTFIL
  ₺310       ₺320       ₺330
```

FIG. 12

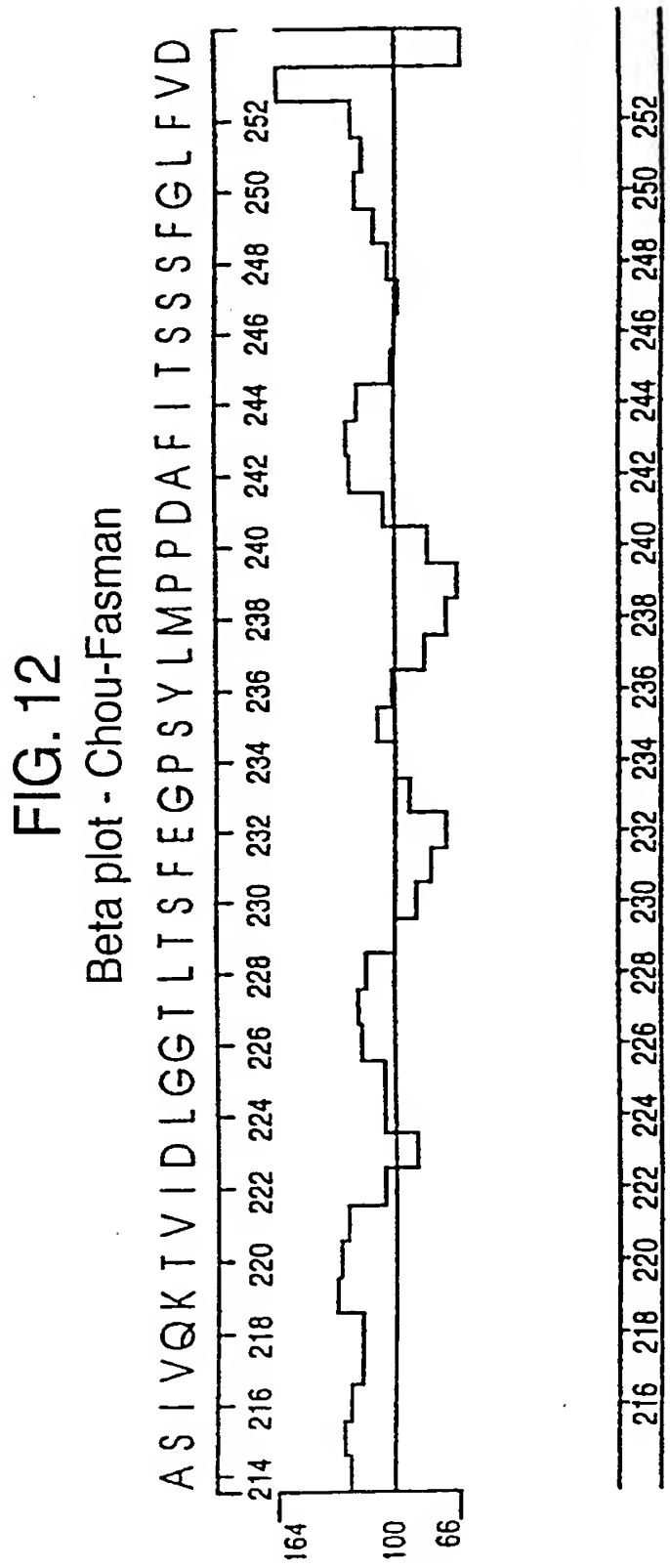

Beta plot - Chou-Fasman
A S I V Q K T V I D L G G T L T S F E G P S Y L M P P D A F I T S S S F G L F V D
214 216 218 220 222 224 226 228 230 232 234 236 238 240 242 244 246 248 250 252
164
100
66
216 218 220 222 224 226 228 230 232 234 236 238 240 242 244 246 248 250 252

FIG. 14(a)

Sequence of RCNMV Coat Protein Spanning The Potential Insertion Site With Introduced Base Changes and New Restriction Sites: (sequence starts at nt 3070)

S I V Q K T V I D L G G T L T S F

AGCATCGTACAGAAAACTGTAATTGATCTCGGTGGGACACTCACTTCTTTC

GTGCAC — *ApaLI*

GTTAAC — *HpaI*

FIG. 14(b)

Series of Sequences to be Inserted Between the Restriction Sites to Insert the MUC1(16) Epitope at Various Locations

G V T S A P D T R P A P G S T A

GGTGTTACTTCTGCTCCTGATACTAGACCTGCTCCTGGTTCTACTGCT

CCACAATGAAGACGACCACTATGATCTGGACGAGGACCAAGATGACGA

GAAAACTGTA	ATTGATCTCGGTGGGACGTT
ACGTCTTTTGACAT	TAACTAGAGCCACCCTGCAA
GAAAACTGTAATT	GATCTCGGTGGGACGTT
ACGTCTTTTGACATTAA	CTAGAGCCACCCTGCAA
GAAAACTGTAATTGAT	CTCGGTGGGACGTT
ACGTCTTTTGACATTAACTA	GAGCCACCCTGCAA
GAAAACTGTAATTGATCTC	GGTGGGACGTT
ACGTCTTTTGACATTAACTAGAG	CCACCCTGCAA
GAAAACTGTAATTGATCTCGGT	GGGACGTT
ACGTCTTTTGACATTAACTAGAGCCA	CCCTGCAA
GAAAACTGTAATTGATCTCGGTGGG	ACGTT
ACGTCTTTTGACATTAACTAGAGCCACCC	TGCAA